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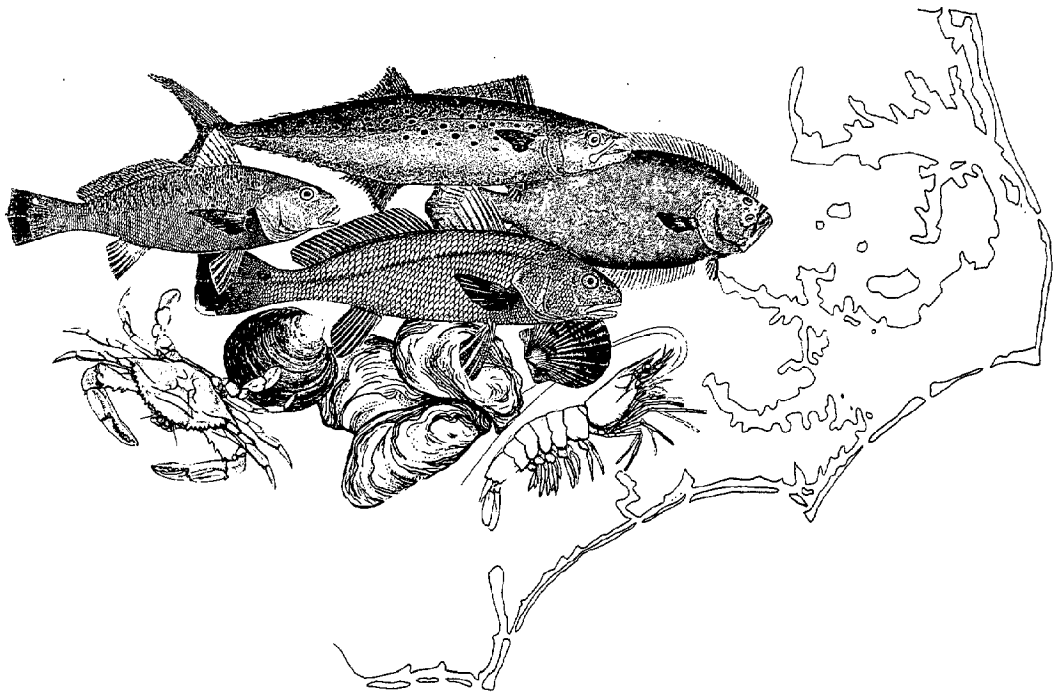

North Carolina Department of Environment,
Health, and Natural Resources

Division of Marine Fisheries
Morehead City, NC 28557

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SHELL DISEASE OF BLUE CRABS, *Callinectes sapidus*, IN THE PAMLICO RIVER, NORTH CAROLINA

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ABSTRACT

Blue crabs with large lesions on their carapace were collected in the Pamlico River estuary during the summer and fall of 1987. Diseased crabs were most abundant on the south side of the River between Durham and South creeks. Infection studies showed a significant difference in the amount of time required for lesion development between this area and control sites. Chitin-degrading and lipolytic bacteria were cultured from natural and experimentally induced lesions. The etiology of shell disease within the Pamlico River estuary is probably stress due to a change in environmental quality. Possible stress-related events include abnormal shell synthesis, abnormal shell repair or degradation of the shell in the water column.

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INTRODUCTION

Shell disease is a ubiquitous syndrome expressing itself as necrotic lesions on the exoskeleton of aquatic crustaceans (Rosen 1970; and Fisher et al. 1976). This disease has been observed in the American lobster (Homarus americanus) (Hess 1937; Young and Pearce 1975; Fisher et al. 1976; Malloy 1978), European lobster (Homarus gammarus) (Roald et al. 1981), blue crab (Callinectes sapidus) (Rosen 1967; Cook and Lofton 1973; Sandifer and Eldridge 1974; Overstreet, 1978; Johnson 1983), tanner crab (Chionoecetes tanneri) (Baross et al. 1978), rock crab (Cancer irroratus) (Young and Pearce 1975), penaeid shrimp (Cook and Lofton 1973; Johnson 1978; Overstreet 1979; Cipriani et al. 1980), freshwater shrimp (Macrobrachium spp. and Palaemonetes spp.) (Johnson 1977), crawfish (Procambarus spp.) (Johnson 1977), caridean shrimp (Crangon septemspinosa (Gopalan and Young 1975), and brown shrimp (Crangon crangon) (Nottage 1982).

The early stage of shell disease manifests itself as numerous punctiform brown marks with reddish-brown depressed centers (Rosen 1970). As the disease progresses, these lesions deepen and eventually coalesce (Johnson 1983). The edges of the lesions may be black or rimmed in blue, presumably due to melanin deposition (Johnson 1983). In natural, unstressed environments, the prevalence of shell disease is low (Hess 1937; Cook and Lofton 1973). However, in degraded environments (Young and Pearce 1975; Gopalan and Young 1975) and under crowded conditions such as holding pens and shedding boxes, the prevalence increases (Rosen 1970; Cook and Lofton 1973; Mallory 1978). The contagious nature of this disease poses a threat to blue crab shedding operations (Rosen 1967; Sandifer and Eldridge 1974). Although all sizes of animals are affected, the more severe cases are usually observed in adults, which may be due to the shorter intermolt period of juveniles (Sandifer and Eldridge 1974; Baross et al. 1978), as the disease is not transferred to the new exoskeleton during ecdysis (Rosen 1970).

Although the etiology of shell disease is uncertain, chitinoclastic bacteria (Vibrio spp., Beneckea spp. and Pseudomonas spp.) are most often associated with the lesions (Hess 1937; Rosen 1967; Cook and Lofton 1973; Malloy 1978). Myxobacteria (Anderson and Conroy 1968), psychrophilic luminescent bacteria, Photobacterium spp. (Baross et al. 1978) and fungi (Rosen 1970) have also been isolated. The polyphenolic epicuticle must be breached in order for chitinoclastic bacteria to attack chitin (Johnson 1983). Rosen (1970) hypothesized that the only way for bacteria to enter was through breaks in the epicuticle. However, Baross et al. (1978) believed that microbial degradation of the epicuticle could be another portal of entry. Lipolytic bacteria may also play an important role in the penetration and removal of the epicuticle (Cipriani et al. 1980).

Recently, blue crabs collected from the Pamlico River, North Carolina have been observed with large lesions on their carapace. The first such animal was caught between Long and Hickory Points on 11 June 1987 (Figure 1). Subsequent fishery dependent sampling by the Division of Marine Fisheries (DMF) indicated a trend to the geographic distribution of these animals. All were caught on the south side of the River between Durham and South Creeks.

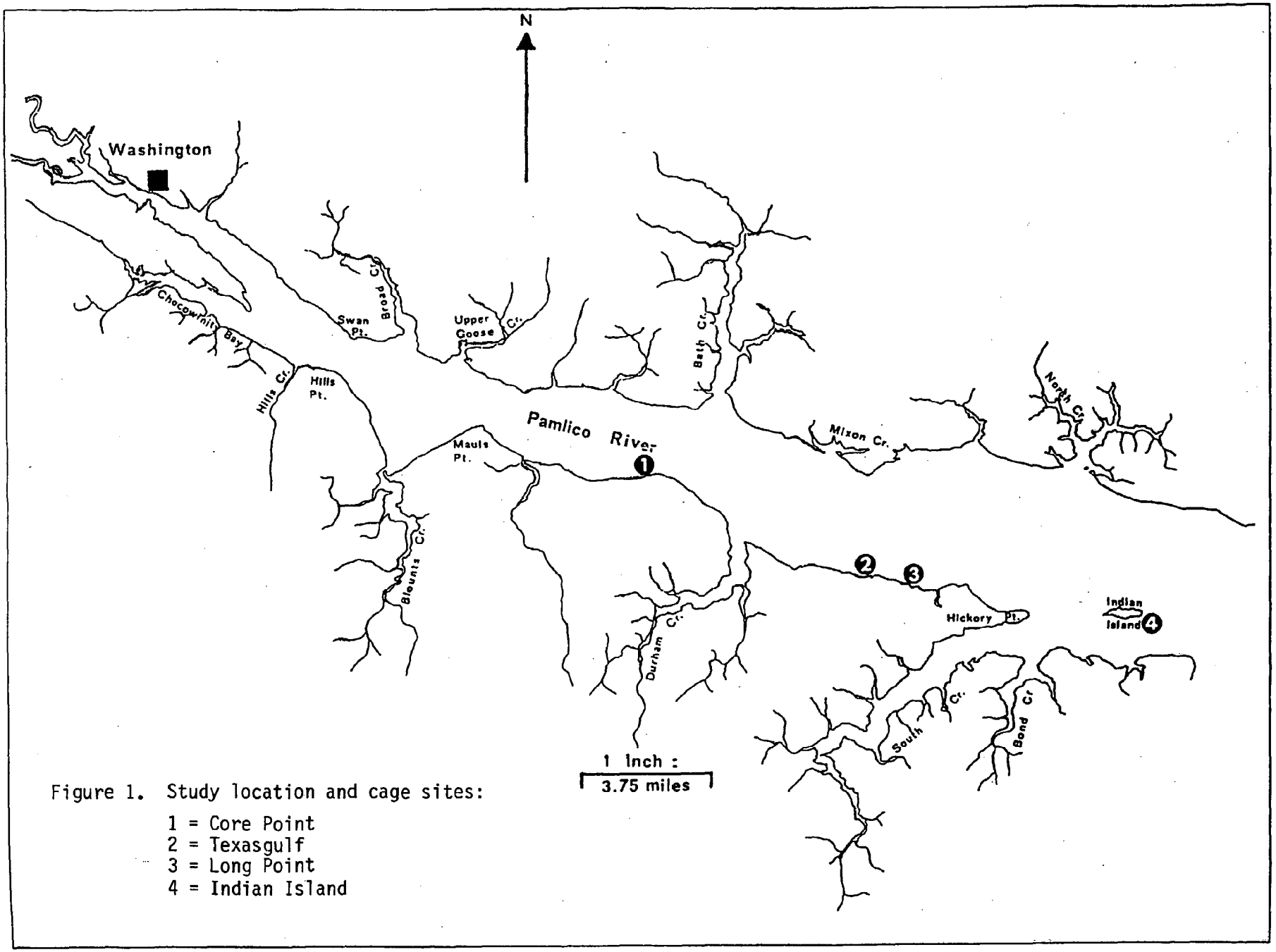

Figure 1. Study location and cage sites:

1 = Core Point
2 = Texasgulf
3 = Long Point
4 = Indian Island

The Pamlico River is one of the largest estuaries in North Carolina and contributes significantly to the state's commercial shellfish and finfish landings. Additionally, this area is used extensively by recreational boaters and fishermen. In recent years the Pamlico River has been the site of numerous fish kills, algae blooms, anoxia events and outbreaks of fish disease (ulcerative mycosis) (Dykstra et al. 1986; Rader et al. 1987). Although the etiologies of these events are uncertain, they might be indicative of declining water quality in the Pamlico. Some of the factors possibly contributing to the decline include freshwater runoff, erosion and sedimentation, nutrients, heavy metals, salinity and dissolved oxygen. An excellent review of these factors is provided by Rader et al. (1987). The addition of shell disease to the problems of the Pamlico River is of great concern to State agencies and the public in general. Not only does shell disease exemplify the deterioration of the water quality in the Pamlico River but, it serves notice that the problems of the Pamlico are far greater than previously perceived.

MATERIALS AND METHODS

The data reported in this paper were obtained from four sources: (1) a trawl survey, (2) a commercial catch sampling program, (3) a sentinel study and (4) bacterial and histological studies. Methods and results are described separately.

Trawl Survey

The objectives of this survey were to: 1) determine the geographic distribution of diseased crabs, 2) investigate the magnitude of infection and 3) determine the distribution of disease in the population.

On 17 July 1987, 60 stations were sampled from Mauls Point to Indian Island using a 6.1 m flat trawl with 19.0 mm bar mesh in the wings and body and 6.4 mm bar mesh in the tail bag. Tow durations were five minutes at each station. Trawls were calibrated to travel 69 m in one minute. A systematic sampling design was employed with the River being divided into grids of 1.3 square kilometers. All crabs caught were measured spike to spike (CW), sexed, maturity noted in females and examined for disease.

Commercial Catch Sampling

To quantify the location of disease on the exoskeleton, 58 crabs captured by commercial fishermen were examined. Forty were randomly collected on 7 July 1987 and 18 on the 10th of July. All crabs were measured (CW), sexed, maturity noted in females and the location of lesions were noted (Figure 2).

Sentinel Study

The purpose of the sentinel study was to determine if "healthy" crabs developed shell disease when placed in the River and if we could differentiate high-risk from low-risk sites for disease development. In addition, we wished to obtain information on infection rates, progression of the disease and environmental factors associated with the disease. Cages were constructed out of 2.5 cm x 1.3 cm vinyl coated hardware cloth and measured 127 cm l x 50.8 cm w x 60.9 cm h. Each cage contained ten individual compartments measuring 25.4 cm l x 25.4 cm w x 60.9 cm h. Cages were placed on the bottom in depths ranging from 0.9 to 1.8 meters. Core Point and Indian Island were in the apparently low risk region, while the Texasgulf effluent and Long Point locations were in the apparently high risk

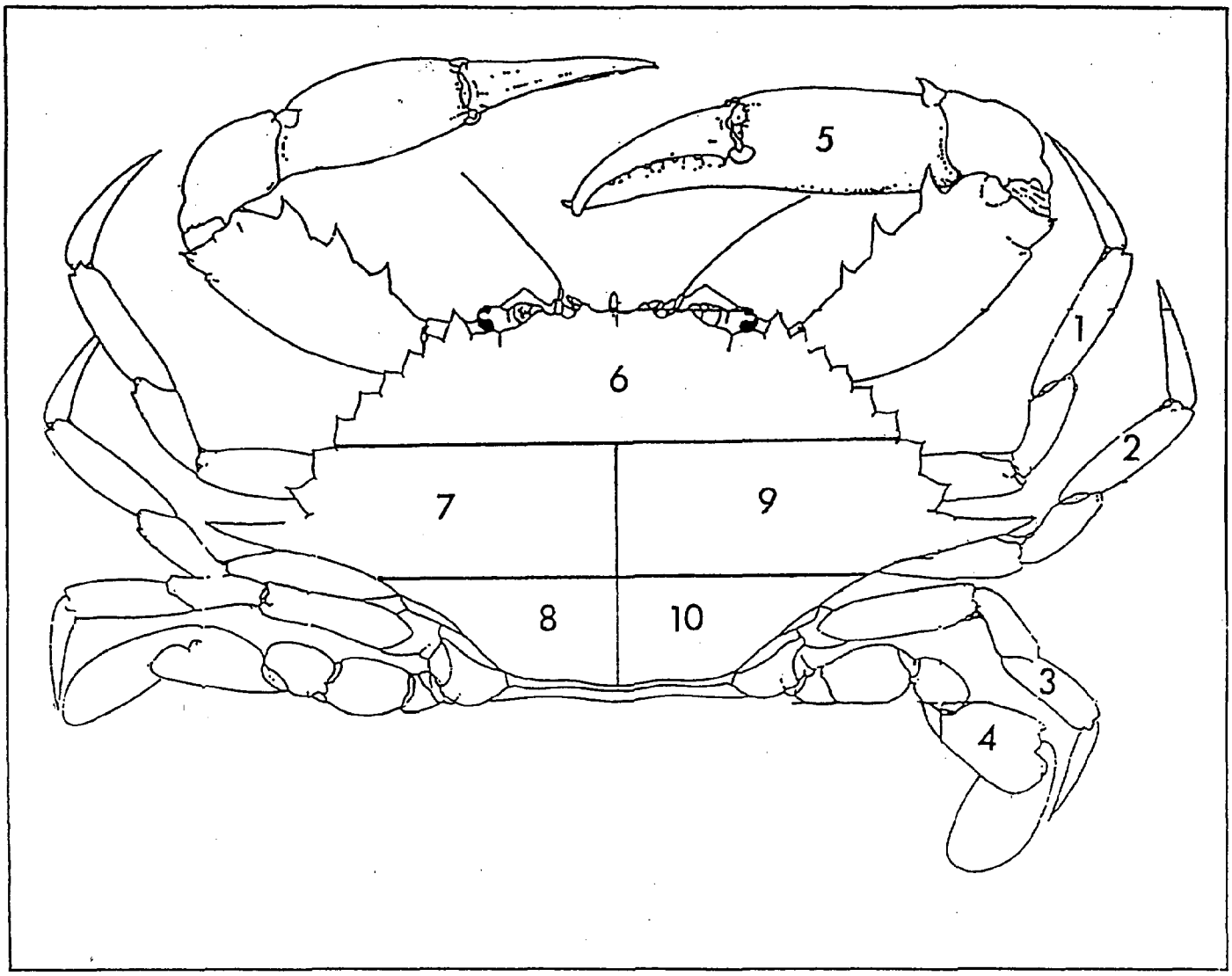

Figure 2. Regions of blue crab exoskeleton.

region (Figure 1). Two cages containing 10 crabs apiece were placed at each site.

Experiment 1

Blue crabs were collected in Rose Bay from commercial crab pot catches (Figure 3). All were mature males measuring 130-160 mm CW. Crabs were placed in cages on 3 August 1987. On 4 August all crabs were measured and any extraneous marks on their exoskeletons were noted. The following day, the epicuticle of five randomly selected crabs from each cage was removed with a knife. All individuals were scraped on the left side over the epibranchial and hepatic region (Figure 4). Weather permitting, crabs were checked daily, fed ad lib with Atlantic menhaden (Brevoortia tyrannus) and any type of mark or discoloration noted. Surface and bottom temperature, salinity and dissolved oxygen were recorded at each station. Initially, any crab which died was replaced with another individual caught in the DMF juvenile stock assessment program. The replace ment animal was treated similarly to the original animal, i.e., control or scraped.

Experiments 2 and 3

In late August, the above procedure was stopped and replacement animals were treated in one of two ways: 1) removal of the epicuticle and exocuticle or 2) fracturing of the exoskeleton. Blue crabs used in these experiments were collected by crab pots set at each study site; only apparently healthy crabs were used. Animals were marked in the same area as described in experiment 1. A knife was used to remove the epicuticle and exocuticle, while fracturing of the exoskeleton was accomplished by use of a blunt instrument or by allowing another crab to bite the treatment animal. The Mann-Whitney Test was used to examine differences between time required to develop lesions and life expectancy of study animals (Zar 1974).

Bacterial and Histological Studies

On 14 September 1987, 37 crabs were sacrificed for bacterial and histological examinations. Blue crabs used for these examinations were taken from the sentinel study and crab pots set at each location. Crabs were placed in individually labeled bags and kept on ice until their return to the lab. All individuals were measured (CW), sexed and a detailed description of lesions noted. Bacterial cultures were obtained by dipping a 1 ul loop mini tip culturette in a modified salt water yeast extract broth (MSWYE) (0.1% protease peptone, 0.1% yeast extract in a three salt solution of 0.4M NACL, 0.028M MgSO 7H O, 0.01M KCL and distilled water) (Cipriani et al. 1980), quickly touching the perifery of the lesion and streaking agar plates containing salt water yeast extract (SWYE) (1.0% protease peptone, 0.3% yeast extract, 2.0% agar 0.4M NACL, 0.028M MgSO 7H O and 0.01M KCL), (ibid). Plates were incubated at room temperature and the number of colonies noted after 24 and 48 hours. Before streaking the agar plates, the culturettes were tapped onto sterile microscope slides in order to obtain gram stains. Slides were then heat fixed. The lesion was then excised and placed in Bouins fixative. Shells were prepared for histological examination by fixation in neutral buffered formalin, decalcified in formic acid and stained with hematoxylin and eosin (H+E).

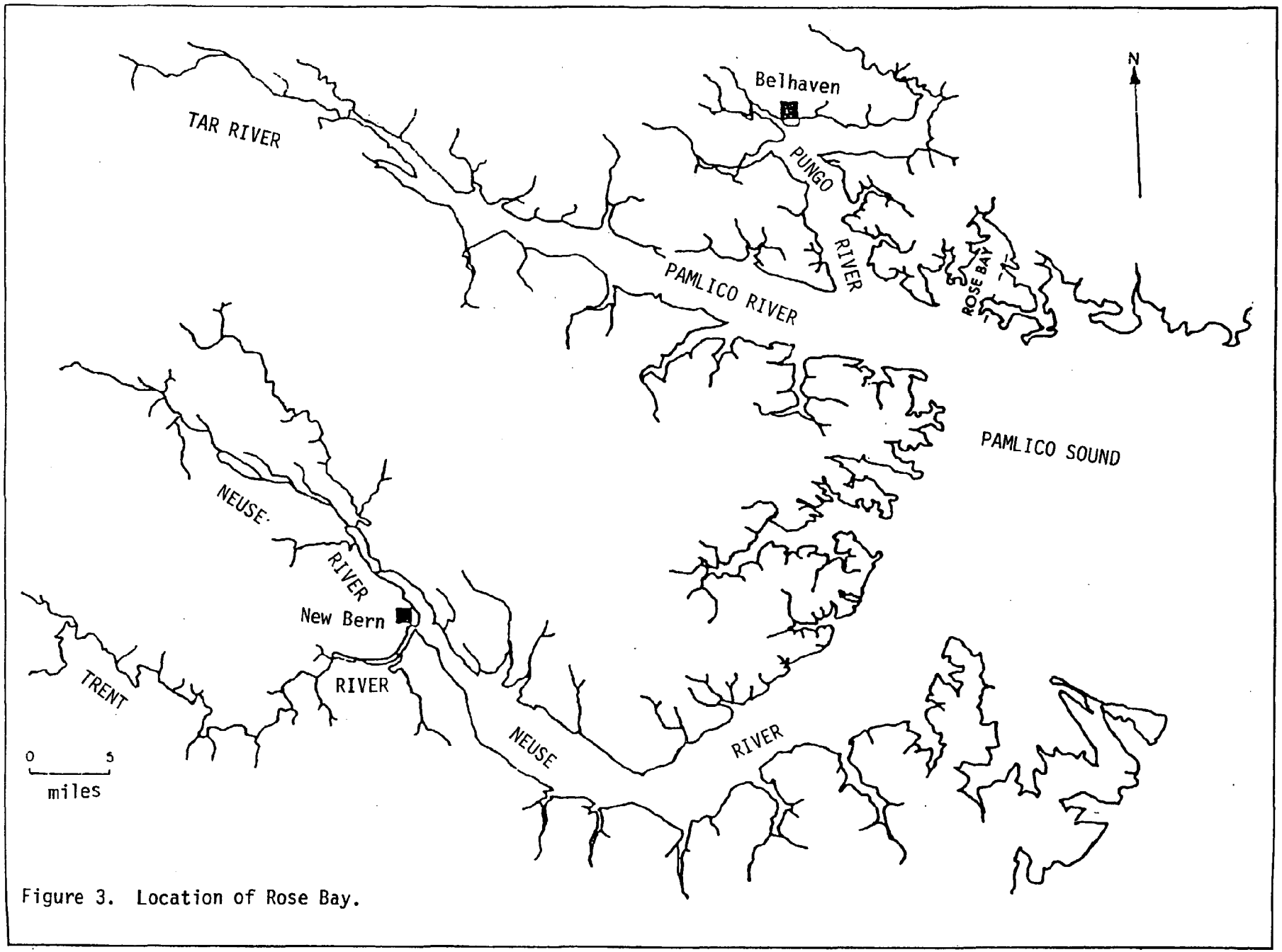

Figure 3. Location of Rose Bay.

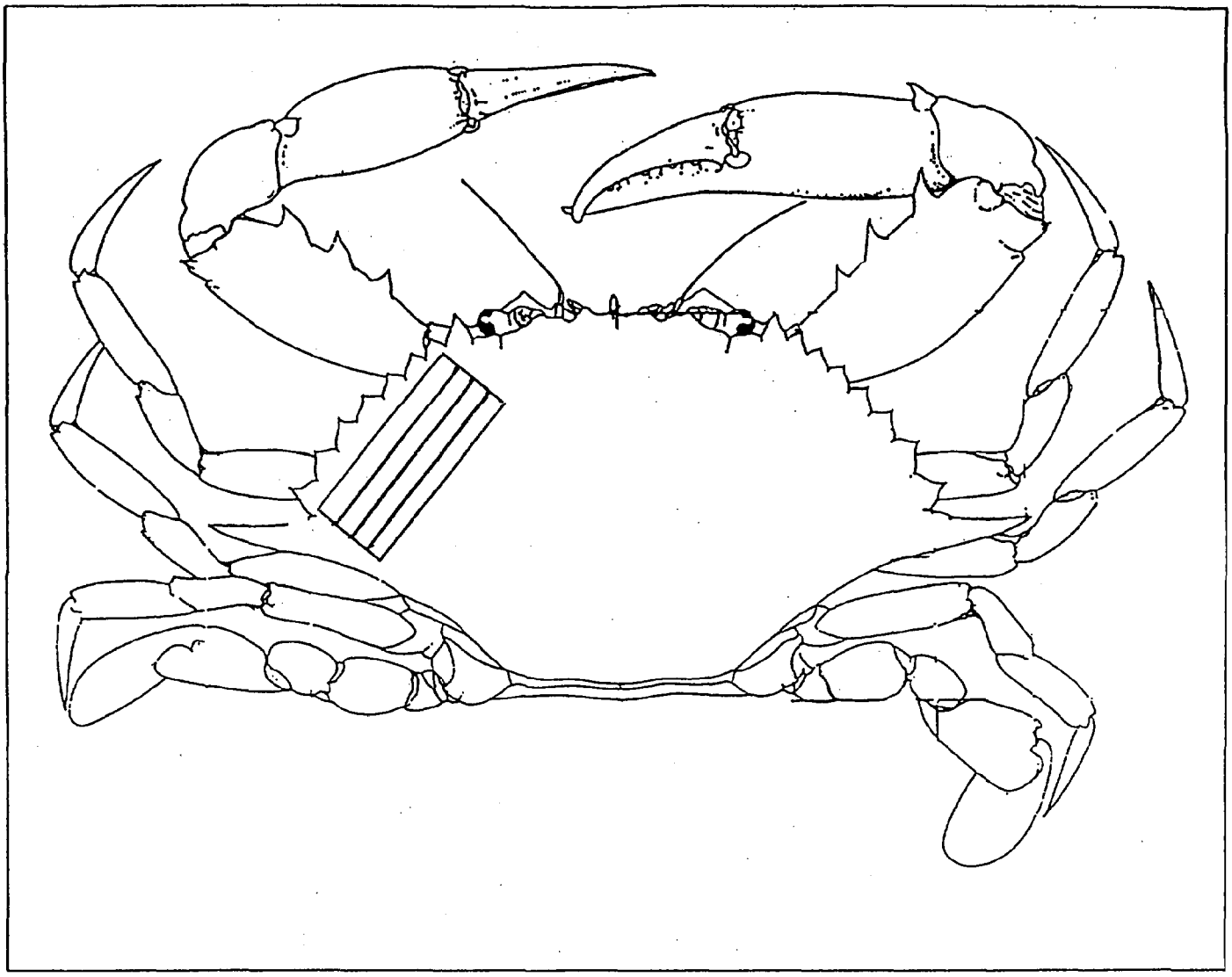

Figure 4. Location of area either scraped or damaged for Experiments 1 - 3.

RESULTS

Trawl Survey

A total of 1,459 blue crabs was examined during the course of this investigation (Tables 1 and 2). Seventy-three (5.0%) diseased crabs were found. The infection rate for males was 5.1%, 2.5% for immature females and 16.2% for mature females. The highest prevalence of disease occurred on the south side of the River, where 16 of the 26 stations (62.0%) had diseased crabs (Figure 5). Ninety-four percent of these stations were located between Durham Creek and Indian Island. Five (31.2%) of the 16 stations located on the north side of the river had diseased crabs. There was a strong relationship between the number of crabs caught and water depth. Sixty-nine percent were caught in water less than 1.8 meters, 30.3% in water 1.8 to 3.6 meters and .4% in greater than 3.6 meters. The same trend held true for diseased crabs, with 72.5% caught in the shallowest strata, 27.4% caught in the 1.8 to 3.6 meter range, while none were caught in the deep water (Table 3).

Commercial Catch Sampling

Of the 57 diseased crabs examined, 28 were males and 29 were mature

Table 1. Standard statistics for healthy blue crabs captured by trawl 17 July 1987 in Pamlico River, North Carolina.

Sex	No.	$\bar{x}$ CW (mm)	SD	CV	Range (mm)	Percent of total
Males	938	112.01	35.66	31.83	26-203	67.67
Immature females	386	88.80	29.28	32.97	31-162	27.84
Mature females	62	157.93	11.68	7.39	122-185	4.47

Table 2. Standard statistics for diseased blue crabs captured by trawl 17 July 1987 in Pamlico River, North Carolina.

Sex	No.	$\bar{x}$ CW (mm)	SD	CV	Range (mm)	Percent of total
Males	51	134.37	28.48	21.10	72-195	69.86
Immature females	10	94.60	26.40	27.91	62-132	13.69
Mature females	12	155.40	13.32	8.57	138-183	16.43

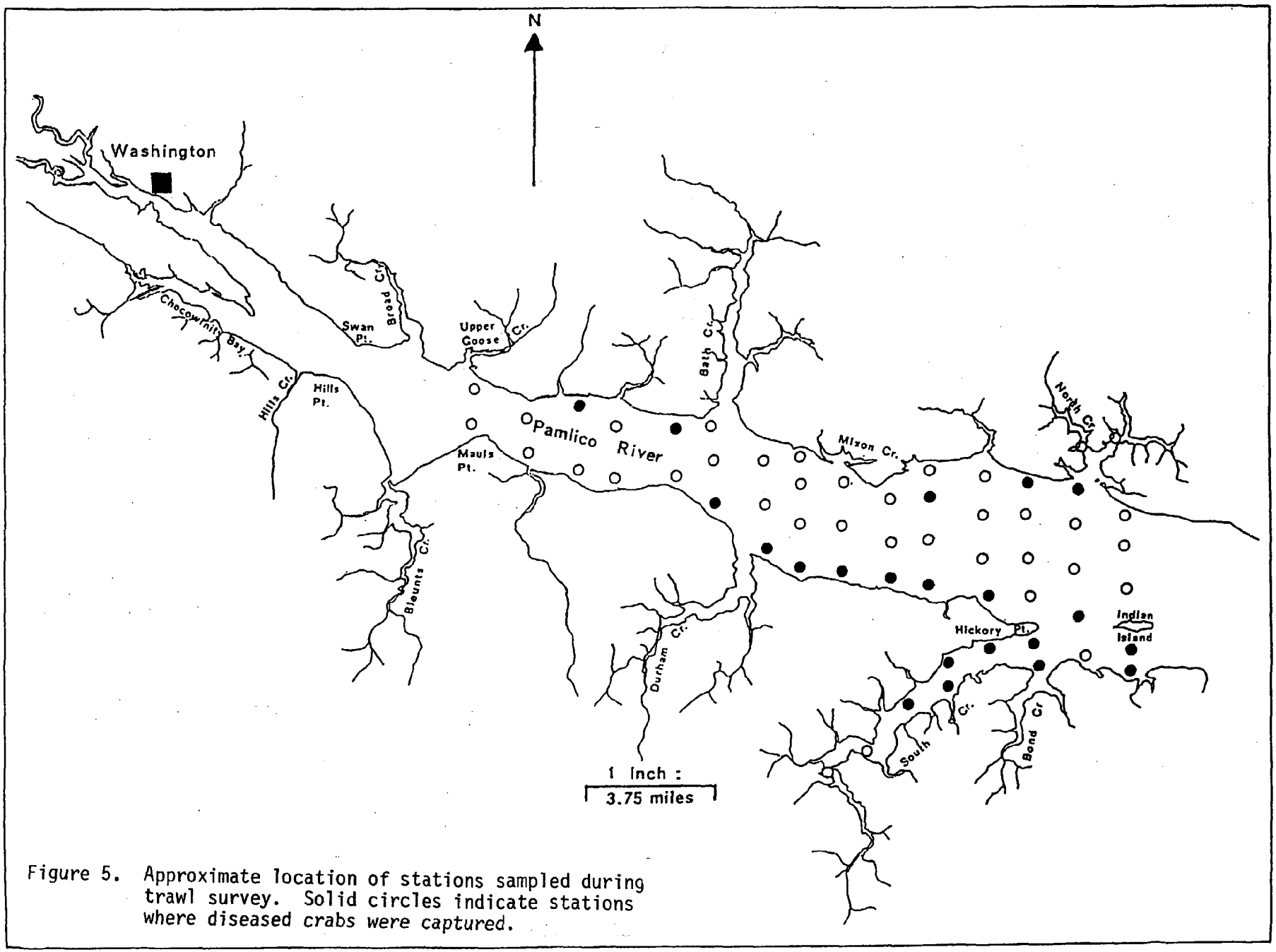

Figure 5. Approximate location of stations sampled during trawl survey. Solid circles indicate stations where diseased crabs were captured.

Table 3. Relationship between water depth and the prevalence of healthy and diseased crabs in the Pamlico River, North Carolina.

Depth strata (m)	Total number of stations	Total number of crabs	Total number without disease	Total number with disease	CPUE[1] without disease	CPUE[1] diseased
0 - 1.82	25	1,022	969	53	7.75	0.42
1.83 - 3.65	22	447	427	20	4.06	0.18
>3.66	13	6	6	0	0.09	0.00

[1]CPUE - catch per unit effort where one minute sample time = one unit of effort.

females. The mean CW for males was 141.3 mm and 159.5 mm for mature females. All animals had lesions on the dorsal surface of the carapace. Four males and no mature females had lesions on the ventral surface. Dorsally, the highest percentage of lesions was in area 6 for both males and females, 42.4% and 47.2% respectively. This was followed by area 7 (33.3% males, 22.2% females), area 9 (18.2% males, 13.9% females), area 8 (30.3% males, 8.3% females) and area 10 (3.0% males, 8.3% females). Three males had lesions on the chela (1 right, 2 left) and five females were affected (3 right, 2 left). With the exception of a mature female, all animals had unaffected walking legs. The affected female had a lesion on the propodite of the fourth walking leg.

No multiple lesions were noted on the ventral surface. One animal had a small lesion on the 5th abdominal segment, another below the right eye, the third in the subhepatic region and the last animal below the mouth. Five males and six females had multiple lesions on the dorsal surface. Three of the males had lesions in both areas 6 and 7, while one individual had lesions in areas 6 and 9 and the last in areas 7 and 9. Five of the six females had lesions in area 6. Four of those had an additional lesion in areas 7, 8, 9 or 10. The other animal with a lesion in area 6 was also affected in areas 7, 8 and 10. The last animal was affected in areas 7, 9 and 10.

Sentinel Study

The results of Experiment 1 are shown in Tables 4-7. Of the ten crabs scraped at Indian Island, seven developed the disease. The mean number of days required for initiation of the disease was 8.7 (SD 6.3). The mean survival time for crabs held at Indian Island was 26.2 days. All of the scraped crabs held at Long Point and Texasgulf developed the disease. The mean number of days required for initiation of the disease was 3.8 (SD 2.89) at Long Point and 4.7 (SD 3.27) at Texasgulf. The mean survival time for Long Point was 27.5 days and 14.7 for Texasgulf. Nine of the ten crabs at Core Point developed the disease. The average number of days required for development of the disease was 7.7 (SD 1.8). Crabs at this location lived

Table 4. Results of experiment 1 for blue crabs near Indian Island, Pamlico River, North Carolina.

Crab & cage no.	Control	Scraped	Number of days alive*	No. of days for first sign of disease*		No. of days for pitting*	
				Control	Marked	Control	Marked
1 - 1		X	10A		3		NP
2 - 1	X		17B	NS		NP	
3 - 1	X		55B	NS		NP	
4 - 1		X	10B		NS		NP
5 - 1	X		24B	NS		NP	
6 - 1		X	17B		10		NP
7 - 1	X		32B	NS		NP	
8 - 1	X		49B	NS		NP	
9 - 1		X	31B		NS		NP
10 - 1		X	41B		21		NP
1 - 2		X	1B		NS		NP
2 - 2	X		13B	NS		NP	
3 - 2		X	19B		6		NP
4 - 2	X		40C	NS		NP	
5 - 2	X		3B	NS		NP	
6 - 2		X	40C		10		NP
7 - 2	X		59C	NS		NP	
8 - 2		X	55B		9		36
9 - 2	X		27A	NS		NP	
10 - 2		X	10A		2		NP

* = A: escaped
B: died
C: terminated
NS: no sign of disease
NP: no pitting

Table 5. Results of experiment 1 for blue crabs near Long Point, Pamlico River, North Carolina.

Crab & cage no.	Control	Scraped	Number of days alive*	No. of days for first sign of disease*		No. of days for pitting*	
				Control	Marked	Control	Marked
1 - 3	X		37B	NS		NP	
2 - 3		X	15B		1		NP
3 - 3		X	55B		1		33
4 - 3		X	15A		6		NP
5 - 3	X		38B	NS		NP	
6 - 3	X		59C	NS		NP	
7 - 3		X	28B		6		NP
8 - 3	X		40C	NS		NP	
9 - 3		X	24B		8		NP
10 - 3	X		13B	NS		NP	
1 - 4	X		1B	NS		NP	
2 - 4		X	9B		2		NP
3 - 4		X	51B		2		NP
4 - 4	X		40B	NS		NP	
5 - 4		X	26B		8		NP
6 - 4		X	32B		1		NP
7 - 4	X		26B	NS		NP	
8 - 4	X		31B	NS		NP	
9 - 4	X		14B	NS		NP	
10 - 4		X	28B		3		NP

* = A: escaped
B: died
C: terminated
NS: no sign of disease
NP: no pitting

Table 6. Results of experiment 1 for blue crabs near Texasgulf effluent station, Pamlico River, North Carolina.

Crab & cage no.	Control	Scraped	Number of days alive*	No. of days for first sign of disease*		No. of days for pitting*	
				Control	Marked	Control	Marked
1 - 5	X		1A	NS		NP	
2 - 5		X	59C		5		35
3 - 5	X		3B	NS		NP	
4 - 5	X		24B	NS		NP	
5 - 5		X	17B		1		NP
6 - 5		X	40C		5	NP	
7 - 5	X		21B	NS		NP	
8 - 5		X	12B		5		NP
9 - 5		X	10A		3		NP
10 - 5	X		6B	NS		NP	
1 - 6	X		23B	NS		NP	
2 - 6		X	12A		10		NP
3 - 6		X	33B		7		22
4 - 6	X		40C	NS		NP	
5 - 6		X	40C		1		38
6 - 6		X	3B		1		NP
7 - 6	X		15B	NS		NP	
8 - 6	X		8B	NS		NP	
9 - 6		X	12B		9		NP
10 - 6	X		7A	NS			

* = A: escaped
B: died
C: terminated
NS: no sign of disease
NP: no pitting

Table 7. Results of experiment 1 for blue crabs near Core Point, Pamlico River, North Carolina.

Crab & cage no.	Control	Scraped	Number of days alive*	No. of days for first sign of disease*		No. of days for pitting*	
				Control	Marked	Control	Marked
1 - 7	X		10A	NS		NP	
2 - 7		X	19B		10		NP
3 - 7		X	7B		NS		NP
4 - 7	X		28B	NS		NP	
5 - 7	X		15B	NS		NP	
6 - 7		X	20B		7		NP
7 - 7		X	53B		7	26	
8 - 7	X		20B	NS		NP	
9 - 7	X		15B	NS		NP	
10 - 7		X	35B		9		NP
1 - 8		X	53B		9	26*	
2 - 8	X		8B	NS		NP	
3 - 8	X		12B	NS		NP	
4 - 8		X	26B		5		NP
5 - 8		X	26B		5		NP
6 - 8		X	40C		9		35
7 - 8	X		27B	NS		NP	
8 - 8		X	46B		8		27**
9 - 8	X		40B	NS		NP	
10 - 8	X		11B	NS		NP	

* = A: escaped
B: died
C: terminated
NS: no sign of disease
NP: no pitting
*20 days from start of pitting to healing
**8 days from start of pitting to healing

an average of 25.6 days. The above data reflects the early stages of the disease, i.e. punctiform brown marks with reddish-brown depressed centers. There was a significant ($P<0.05$) difference between the time required for lesion development in the high risk area ($\bar{x}$ = 4.2 days) and the low risk area ($\bar{x}$ = 8.1 days). Due to small sample size, analysis between individual sites was not attempted. There were no significant differences between the mean survival time of control or scraped crabs (Table 8). Due to the short cage life of crabs, the progression of the disease could not be followed for all individuals. The last column of Tables 4-7 represents the time required for deepening and joining of early lesions. Only one crab at both Indian Island and Long Point developed the latter stages of the disease. The time required for pitting was 36 days at Indian Island and 33 days for Long Point. Three crabs at Texasgulf developed the latter stages of the disease in an average of 31.6 days. At Core Point, pitting occurred on average in 28.6 days (N = 4).

The results of experiment 2 are summarized in Tables 9-12. Only crab 3, cage 5 developed lesions similar to ones seen in the high risk area. This involved the complete breakdown of the crystalline matrix of the endocuticle. Eleven days later new "wound cuticle" (Neville 1975) was deposited. Two other animals at the Texasgulf location exhibited endocuticle breakdown (crabs 4 and 10, cage 5), but they were either terminated for bacteriological examination (crab 10) or died (crab 4) before healing occurred.

Experiment 3 involved the fracturing of the exoskeleton. The objective of this experiment was to examine the healing process. A total of 20 animals were treated: Indian Island (3), Long Point (6), Texasgulf

Table 8. Results of Mann-Whitney test for comparisons of mean survival time or blue crabs sentinel experiment in Pamlico River, North Carolina.

Comparison	U	U'	Significance level
Low risk area[1] control vs scraped	153.5	102.5	NS[2]
High risk area[3] control vs scraped	87.0	123.0	NS
Control low vs high risk area	132.5	107.5	NS
Scraped low vs high risk area	127.0	97.0	NS

1 Core Point and Indian Island
2 Not significant at the 0.05 level
3 Texasgulf and Long Point

Table 9. Results of experiment 2 for blue crabs near Indian Island, Pamlico River, North Carolina.

Crab and cage no.	Date scraped	Number of days required to* note changes in scraped area					Days alive
		Yellow	Yellow-Brown	Brown	Black	Pitting	
9 - 2	9/1	NS	2	6	NS	8	13C
4 - 1	9/3	NS	NS	NS	7	NS	11C
9 - 1	9/5	2	NS	NS	NS	NS	4B
1 - 1	9/7	4	NS	NS	4	NS	8 shed
7 - 1	9/7	NS	NS	NS	NS	NS	2 shed
9 - 1	9/10	NS	NS	NS	NS	NS	4C

* = A: escaped
B: died
C: terminated
NS: no sign of disease

Table 10. Results of experiment 2 for blue crabs near Long Point, Pamlico River, North Carolina.

Crab and cage no.	Date scraped	Number of days required to* note changes in scraped area			Days alive
		Yellow	Brown	Pitting	
5 - 4	8/31	1	NS	NS	5B
9 - 4	8/31	1	NS	NS	5B
5 - 4	9/5	2	NS	4	12B
7 - 4	9/5	2	NS	4	12B
1 - 4	9/6	NS	3	5	27C
7 - 3	9/6	1	NS	6	19B
9 - 3	9/7	2	NS	5	10B
2 - 4	9/10	1	NS	2	4C
9 - 4	9/12	NS	NS	NS	5C

* = A: escaped
B: died
C: terminated
NS: no sign of disease

Table 11. Results of experiment 2 for blue crabs near Texasgulf, Pamlico River, North Carolina.

Crab and cage no.	Date scraped	Number of days required to* note changes in scraped area						Days alive
		Yellow	Brown	Pitting	Breakdown of crystalline matrix	Healing	Blue	
7 - 5	9/1	2	NS	5	NS	NS	NS	13C
3 - 5	9/3	6	NS	NS	3	14	20	29C
4 - 5	9/3	NS	3	3	7	NS	NS	13B
10 - 5	9/7	2	NS	NS	2	NS	NS	7C
3 - 6	9/10	1	2	7	NS	NS	NS	23C
9 - 6	9/10	2	NS	NS	NS	NS	NS	4C
8 - 5	8/29	2	3	8	NS	22	NS	35C

* = A: escaped
B: died
C: terminated
NS: no sign of disease

Table 12. Results of experiment 2 for blue crabs near Core Point, Pamlico River, North Carolina.

Crab and cage no.	Date scraped	Number of days required to* note changes in scraped area					Days alive
		Yellow	Yellow-brown	Brown	Pitting	Healing	
6 - 7	8/28	NS	NS	4	NS	NS	20C
3 - 7	9/1	1	NS	NS	NS	NS	2B
7 - 8	9/1	1	5	8	8	NS	13B
3 - 7	9/3	3	NS	6	6	NS	14C
1 - 7	9/10	1	NS	NS	2	7	23C
10 - 7	9/10	1	NS	7	NS	7	10B

* = A: escaped
B: died
C: terminated
NS: no sign of disease

(6), Core Point (5). The mean time required for deposition of wound cuticle was 24 days Indian Island (N = 2), 25 days Long Point (N = 1), 23 days Texasgulf (N = 1) and 20 days Core Point (N = 5). Crabs with large fractures in which the epidermis was breached never healed completely. Regardless of location, all fractures increased in size. The edges of the fracture became brittle and eventually sloughed off.

Five predominant bacterial colony types were isolated: Type A Pseudomonas spp., Type B unidentified, Type C Vibrio spp., Type D Vibrio spp. and Type E Proteus spp. (Table 13). Bacterial compositions for the various study animals are given in Tables 14-17 and for natural lesions in Table 18.

The three environmental parameters measured (temperature, salinity and dissolved oxygen) are summarized in Table 19.

DISCUSSION

There appeared to be a trend in the geographic distribution of diseased crabs in the Pamlico River. The trend first became evident through interviews with local fishermen and was substantiated with results of the trawl survey. Throughout the course of this investigation, fishermen were interviewed daily as to the location of diseased crabs in their catch. For the most part, the capture of diseased crabs was limited to the area between Durham and South Creeks. Occasionally diseased crabs were caught outside of this area, but within the Pamlico River estuary. The low frequency of these reports were attributed to random movements (Judy and Dudley 1970; Sholar 1983). Blue crabs with lesions as severe as those seen in the Pamlico River were examined from Masonboro, Bogue and Core Sounds. The total number of crabs examined was eight and in all cases the damage was attributed to mechanical

Table 13. Description of the predominant bacterial colonies isolated from experimental crabs collected in Pamlico River, North Carolina during September, 1987.

	Bacteria type				
	A	B	C	D	E
Gram reaction	-	-	-	-	-
Shape	rod	stippled rod	curved rod	rod	rod
Color on SWYE agar	orange	white	opaque white	translucent white	white

Table 14. Number of bacterial colonies isolated from crabs at Indian Island, Pamlico River, North Carolina, September, 1987.

Crab	Bacteria type A	B	C	D	E	Total number of colonies	Description of lesion
1	0	0	0	0	0	0	Scraped 9/3 9/14 1 black spot 1 mm diameter
2	11	20	0	0	4	60	Scraped 9/10 9/14 no color
3	0	0	0	0	1*	1	Scraped 9/1 9/14 brown and little pitting
4	0	0	0	0	0	0	Scraped 8/5 9/14 3 brown spots each 1 mm
5	0	0	0	0	0	0	Scraped 9/12 9/14 no color
6	0	-	-	0	*	*	NAShed 9/9 9/14 multiple rust marks
7	0	0	0	0	0	0	Control
8	0	0	0	0	0	0	Control

* Plate overgrown with type E
** 75% of plate overgrown with type E, 10% with type B and 10% type C
N/A not available

Table 15. Number of bacterial colonies isolated from crabs at Long Point, Pamlico River, North Carolina, September, 1987.

Crab	Bacteria type					Total number of colonies	Description of lesion
	A	B	C	D	E		
1	0	0	0	0	0	0	Scraped 9/12 9/14 10% area yellow
2	48	48	12	0	0	120	Scraped 9/12 9/14 100% area yellow
3	14	14	0	16	1	45	Scraped 9/12 9/14 5% area yellow
4	2	19	5	4	0	29	Scraped 9/10 9/14 85% yellow 15% brown little pitting
5	0	0	0	0	0	0	Control
6	0	0	0	0	0	0	Control

Table 16. Number of bacterial colonies isolated from crabs at Texasgulf outfall, Pamlico River, North Carolina, September, 1987.

Crab	Bacteria type A	B	C	D	E	Total number of colonies	Description of lesion
1	0	0	0	0	1*	1	Scraped 8/5 9/14 mottled brown
2	0	0	0	0	0	0	Scraped 8/5 9/14 little pitting
3	10	0	10	0	60	100	Crab bit 8/29 9/14 edges brown
4	0	0	0	0	1*	0	Scraped 9/12 9/14 50% yellow
5	-	0	-	0	0	TNTC	Scraped 9/12 9/14 85% yellow
6	9	200	12	4	9	300	Scraped 9/1 9/14 10% yellow & pitted 90% brown
7	0	0	0	0	1*	1	Scraped 9/10 9/14 40% yellow
8	0	6	10	0	1	28	Scraped 9/7 9/14 50% brown & soft
9	0	0	0	0	0	0	Control
10	0	0	0	0	0	0	Control
11	0	0	0	0	0	0	Control

* Plate overgrown with type E.
TNTC to numerous to count, estimated that 90% of plate covered by type A + 10% by type C.

Table 17. Number of bacterial colonies isolated from crabs at Core Point, Pamlico River, North Carolina, September, 1987.

Crab	Bacteria type A	B	C	D	E	Total number of colonies	Description of lesion
1	0	0	0	0	1*	1	Scraped 8/5 9/14 brown & pitted
2	8	8	5	9	0	30	Fractured 8/28
3	0	1	1	0	0	2	Scraped 8/28 9/14 area brown
4	70	19	8	0	0	100	Scraped 9/3 9/14 entire area brown 5% pitted
5	15	11	17	34	0	77	Scraped 9/12 9/14 75% yellow
6	16	4	0	0	0	20	Scraped 9/12 9/14 90% area yellow
7	2	20	4	1	0	30	Scraped 9/12 9/14 20% area yellow
8	6	4	9	30	0	60	Scraped 9/12 9/14 20% area yellow
9	0	0	0	0	0	0	Control
10	1	5	6	6	0	20	Control
11	0	0	0	0	0	0	Control
12	0	0	0	0	0	0	Control

*Sparse growth

Table 18. Number of bacterial colonies isolated from natural lesions of blue crabs from Pamlico River, North Carolina, September, 1987.

Crab	Bacteria type A	B	C	D	E	Total number of colonies	Description of lesions
1	5	11	3	23	1	43	Brown pitted
2	5	11	0	0	0	40	Brown & pitted
3	3	20	0	12	0	40	Brown & pitted

Table 19. Monthly temperature, salinity and dissolved oxygen ($\bar{x}$ SD) for the four study sites in Pamlico River, North Carolina, August-September, 1987.

	Temperature		Salinity		Dissolved oxygen	
	August	September	August	September	August	September
Indian Island						
Top	28.05 1.0	26.43 0.8	11.45 1.8	12.66 1.3	6.60 0.9	7.61 1.0
Bottom	28.02 0.9	26.20 1.0	11.47 1.6	12.71 1.2	6.49 0.9	7.50 1.0
Long Point						
Top	28.15 1.2	26.44 1.0	9.90 1.4	11.06 1.5	6.77 1.1	7.60 1.6
Bottom	28.18 1.1	26.04 1.3	10.08 1.4	11.06 1.5	6.33 0.9	7.09 1.5
Texasgulf						
Top	28.29 1.6	26.63 1.5	8.22 1.8	9.66 2.0	6.64 1.2	6.89 0.7
Bottom	28.30 1.5	26.38 1.4	8.93 1.5	10.11 1.4	6.44 1.5	6.91 1.1
Core Point						
Top	28.65 1.7	26.54 1.9	8.15 1.0	8.63 1.5	7.01 1.2	7.23 1.1
Bottom	28.37 1.5	26.10 1.7	8.26 1.0	8.85 1.5	6.21 1.1	6.17 1.0

damage, i.e., crab bite. Observation of crabs collected in monthly juvenile sampling by the Pamlico District indicated a widespread distribution of blue crabs with shell disease. However, in no case did the severity of lesions compare to those in the study area. Lesions were located on the appendages and the ventral surface and were more typical of lesions described by Rosen (1970).

The prevalence of shell disease was greatest in larger males and mature females, which probably related to a longer intermolt period for the larger males. Mature females do not shed after their terminal molt (Milikin and Williams 1984). Therefore, they are unable to rid themselves of the disease through ecdysis (Sandifer and Eldridge 1974; Baross et al. 1978). Other factors possibly contributing to the high prevalence of disease in mature females are longevity and the contagious nature of the disease. Van Engel (1958) noted that females can live up to two years following their terminal molt, thus increasing their chance of suffering mechanical damage to the exoskeleton. Due to the contagious nature of the disease and the increased susceptibility of soft crabs to mechanical damage, a female undergoing her terminal molt has a high probability of infection through prolonged contact with its mate especially if the latter is infected.

There are three etiological scenarios for shell disease: stress, mechanical damage and bacteria. Chemical pollutants cause stress in marine animals (Sindermann et al. 1982), often by decreasing an organism's resistance to disease by impairing mechanisms of cellular and humoral immunity (Murchelano 1982). Nottage (1982) hypothesized that subtle changes in body chemistry may influence individual susceptibility to shell disease. Fisher et al. (1976) found that long-term dietary deficiencies were capable of weakening the defenses provided by the epicuticle. Shell disease may be a phenomenom resulting from poor environment conditions found in mariculture raceways (Cipriani et al. 1980), or other heavily fouled waters (Gopalan and Young 1975, Young and Pearce 1975).

The epicuticle of blue crabs contains lipids, proteins and calcium and serves as a first line of defense against microorganisms (Johnson 1980). In vitro inoculation experiments showed that the epicuticle had to be removed in order to create a typical lesion (Bright et al. 1960, McLeese 1965, Cook and Lofton 1973, Cipriani et al. 1980, Nottage 1982). Rosen (1970), Cook and Lofton (1973), Malloy (1978) and Overstreet (1978) hypothesized that mechanical damage to the epicuticle allows chitinoclastic bacteria to enter.

The exoskeleton of crustaceans can be viewed as a microenvironment. Through succession, a primary food source may be utilized by more than one type of bacteria. The waste produced would be utilized by another bacterium with the process being repeated until all possible foods have been used (Gunsalus and Stanier 1962). Cipriani et al. (1980) suggested that lipolytic bacteria might play an important role in the penetration of the epicuticle. Once this layer is breached, then chitinoclastic bacteria might attack the chitin in the exocuticle. The organic acids which are produced as the end-product of chitin digestion would then dissolve the calcium salts (Rosen 1970). These acids might affect calcium deposits in both the epicuticle and exocuticle, which would allow further microbial

degration and lead to the breakdown of the endocuticle.

Regardless of the mechanism for penetration of the epicuticle, the end result is the breakdown of chitin resulting in necrotic lesions. Chitinoclastic bacteria are found world-wide and play an important role in the marine carbon and nitrogen cycles (Cambell and Williams 1951). These bacteria are most abundant in sediments (Hock 1950, Seki 1965, Donderski 1984) where the majority of chitin decomposition occurs (Seki 1965, Hood and Meyers 1977). Therefore, the benthic nature of blue crabs predisposes them to these opportunistic pathogens.

The bacterial fauna identified from natural lesions and study animals were similar, with Pseudomonas spp., Type B bacterium and Vibrio spp. being the predominant organisms present. Pseudomonas spp. and Vibrio spp. have previously been isolated from animals with shell disease (Cook and Lofton 1973, Baross et al. 1978, Malloy 1978, Cipriani et al. 1980, Roald et al. 1981). These findings suggest the role of chitinoclastic bacteria in this shell disease outbreak since these genera often have chitinase activity. The occurrence of Proteus spp. in the bacterial samples has a number of implications. The tendency of this genus to swarm and overgrow the surface of agar plates makes it difficult to isolate other bacteria (Starr et al. 1981). Therefore, quantitative analysis of bacterial compositions between sites was not possible. In aquatic environments, this genus is found in polluted waters (Buchanan and Gibbons 1974, Laskin and Lechevalier 1977). Areas of high organic load and poor water quality generally contribute to an increase in numbers of bacteria (Cook and Lofton 1973, Sindermann 1974), while higher overall bacteria populations generally result in a higher rate of chitin digestion (Hood and Meyers 1977). Organic loading and/or water quality might explain the results of experiment 1, in which the Long Point and Texasgulf crabs developed the early stages of the disease more rapidly than the control sites. Not only did the disease develop more rapidly at these sites, but the severity of infection was greater, especially at the the Texasgulf location.

The various colors of experimental lesions (yellow, brown, black and blue) can be attributed to two factors, melanin deposition and bacteria. Johnson (1983) suggested that darkening of the affected area is due to the deposition of melanin. Malloy (1978) noted that cratering of the lesions on experimental lobsters was accompanied by the development of a yellow slime layer. Whether this is the result of bacterial action is not known, but of the 21 crabs that developed this yellow slime, 14 showed pitting. Roald et al. (1981) noted that lesions were limited to the area within the blue line. This line developed in some animals and was common on crabs with natural lesions.

The lesions of blue crabs caught ouside of the Pamlico River are probably the result of mechanical damage. This damage could be a function of abrasive action, predator-prey relationship or intraspecific interactions, though abrasive action is the most likely explanation. This disease was most prevalent on the appendages and ventral surface. These areas are most often in contact with the sediments and more likely to be damaged through normal activity.

The etiology of shell disease within the Pamlico River estuary is probably stress due to a change in environmental quality. Possible stress-related events include abnormal

shell synthesis, abnormal shell repair or degradation of the shell in the water colum. Brine (1978) examined the chitin content at various locations on the carapace and chelipeds of the blue crab. The dorsal surface of the carapace was divided into seven regions, with percentage of chitin (dry wgt) averaging 15.7%, while the cheliped sections averaged 11.6% chitin. The claws are the defensive instruments of the crab. The reduction in chitin in the claws is a function of higher deposition of calcium salts in the cuticle affording greater structural strength to this appendage (Brine 1978, Greenaway 1985). The epibranchial region has a chitin content of 13.7%, the lowest percentage noted on the dorsal surface of the carapace. This region corresponds in part to area 6 of this study. If a lower chitin content is indicative of an increase in the amount of calcium salt deposition, then this area should be more structurally sound than other areas on the carapace and less likely to suffer mechanical damage. It is interesting to note that crabs caught in the stress zone appeared to have weak carapaces that could easily be broken with slight pressure. The possibility exists that crabs in this area are undergoing abnormal shell synthesis due to a chemical or mineral imbalance resulting in a structurally inferior shell. Thus the shells of these animals may be prone to mechanical damage or bacterial attack. Cadmium and fluroide levels in the Pamlico River were found to be higher along the southside and in particular in the sediments near the discharge of Texasgulf (Barry Adams, pers. comm). Cadmium was found to cause lesions in the carapace of the crab Scylla serrata (D.M. Dhavale, pers. comm.). Arey (1979) noted that high levels of fluoride could tie up calcium ions, which are needed for tanning of the exoskeleton. Future work should examine the effect of these substances and other chemicals on shell synthesis and shell repair.

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